

What is claimed is:

1. A modified neurotoxin comprising:

5 a neurotoxin including a structural modification,
wherein said structural modification is effective to
alter a biological persistence of said modified
neurotoxin relative to an identical neurotoxin without
said structural modification, and wherein said modified
10 neurotoxin is structurally different from a naturally
existing neurotoxin.

15 2. The modified neurotoxin of claim 1, wherein said
structural modification is effective to enhance a
biological persistence of said modified neurotoxin.

3. The modified neurotoxin of claim 1 wherein said
biological persistence of said modified neurotoxin is
reduced relative to an identical neurotoxin without said
structural modification.

20 4. The modified neurotoxin of claim 1 wherein said
structural modification comprises 1 to about 22 amino
acids.

25 5. The modified neurotoxin of claim 1 wherein said
structural modification comprises an amino acid, said
amino acid comprising an R group of 1 to about 12 carbon
atoms.

30 6. The modified neurotoxin of claim 1 wherein said
structural modification comprises a leucine-based motif
(SEQ ID NO: 1).

7. The modified neurotoxin of claim 1 wherein said structural modification comprises a tyrosine-based motif.

5

8. The modified neurotoxin of claim 1 wherein said structural modification comprises an amino acid sequence of a botulinum type A light chain and of an amino acid sequence of a type B light chain.

10

9. The modified neurotoxin of claim 8 wherein said structural modification comprises the amino acid sequence KAFK.

15

10. The modified neurotoxin of claim 8 wherein said structural modification comprises the amino acid sequence YYD in combination with the amino acid sequence YYL in combination with the amino acid sequence T.

20

11. The modified neurotoxin of claim 1 wherein said structural modification comprises an amino acid derivative.

25

12. The modified neurotoxin of claim 1 wherein said neurotoxin is selected from the group consisting of botulinum toxin type A, B, C₁, C₂, D, E, F and G.

13. The modified neurotoxin of claim 1 wherein said neurotoxin is botulinum toxin type A.

30

14. A modified neurotoxin comprising a neurotoxin including a structural modification, wherein said neurotoxin comprises three amino acid sequence regions:

- 5 a) a first region effective as a cellular binding moiety;
- b) a second region effective to translocate a modified neurotoxin or a part thereof across an endosome membrane; and
- 10 c) a third region effective to inhibit exocytosis when released into a cytoplasm of a target cell,

15 wherein at least one of said first, said second and said third regions is substantially derived from a Clostridial neurotoxin, said third region includes said structural modification, a modified neurotoxin is structurally different from a naturally existing neurotoxin, and said structural modification is

20 effective to alter a biological persistence of said modified neurotoxin relative to an identical neurotoxin without said structural modification.

15. The modified neurotoxin of claim 14, wherein said

25 neurotoxin is a member selected from a group consisting of botulinum toxin serotypes A, B, C₁, C₂, D, E, F, G, tetanus toxin and mixtures thereof.

16. The modified neurotoxin of claim 14, wherein said

30 neurotoxin is a member selected from a group consisting of botulinum toxin serotypes A, B, C₁, C₂, D, E, F and G.

17. The modified neurotoxin of claim 14, wherein said neurotoxin comprises botulinum toxin serotypes A.

18. The modified neurotoxin of claim 14, wherein said
5 third region is derived from botulinum toxin serotype A.

19. The modified neurotoxin of claim 14, wherein said third region is not derived from botulinum toxin serotype A.

10

20. The modified neurotoxin of claim 14, wherein said structural modification includes a biological persistence enhancing component effective to enhance said biological persistence of said modified neurotoxin.

15

21. The modified neurotoxin of claim 20, wherein said biological persistence enhancing component comprises a leucine-based motif of SEQ ID NO: 1.

20

22. The modified neurotoxin of claim 20, wherein said leucine-based motif comprises a run of seven amino acids, wherein said run comprises a quintet of amino acids and a duplet of amino acids, wherein said quintet of amino acids defines the amino terminal end of said
25 leucine-based motif and said duplet of amino acids defines the carboxyl end of said leucine-based motif.

25

23. The modified neurotoxin of claim 22, wherein said quintet of amino acids comprises an acidic amino acid,
30 wherein said acidic amino acid is selected from a group consisting of a glutamate and an aspartate.

30

24. The modified neurotoxin of claim 22, wherein said quintet of amino acids comprises a hydroxyl containing amino acid, wherein said hydroxyl containing amino acid is selected from the group consisting of a serine, a threonine and a tyrosine.

25. The modified neurotoxin of claim 24, wherein said hydroxyl containing amino acid can be phosphorylated.

26. The modified neurotoxin of claim 22, wherein said duplet of amino acids comprises at least one amino acid, wherein said amino acid is selected from the group consisting of leucine, isoleucine, methionine, alanine, phenylalanine, tryptophan, valine and tyrosine.

27. The modified neurotoxin of claim 22, wherein said duplet of amino acids is selected from a group consisting of leucine-leucine, leucine-isoleucine, isoleucine-leucine, isoleucine-isoleucine and leucine - methionine.

28. The modified neurotoxin of claim 21, wherein said leucine-based motif comprises an amino acid sequence phenylalanine-glutamate-phenylalanine-tyrosine-lysine-leucine-leucine of SEQ ID NO: 1.

29. A modified neurotoxin wherein said modification comprises a tyrosine-based motif.

30. The modified neurotoxin of claim 29 wherein said tyrosine-based motif comprises a run of four amino acids, wherein an amino acid comprising an N-terminal

end of said run comprises a tyrosine residue and an amino acid comprising a C-terminal end of said run comprises a hydrophobic amino acid.

5 31. The modified neurotoxin of claim 14, wherein said biological persistence of said modified neurotoxin is reduced relative to an identical neurotoxin without said structural modification.

10 32. The modified neurotoxin of claim 31, wherein said structural modification includes a leucine-based motif with mutation to one or more amino acids comprising said leucine-based motif of SEQ ID NO: 1.

15 33. The modified neurotoxin of claim 31, wherein said structural modification includes a tyrosine-based motif with a mutation to one or more amino acids comprising said tyrosine-based motif.

20 34. The modified neurotoxin of claim 31, wherein said structural modification comprises an amino acid derivative with a mutation to one or more amino acids comprising said amino acid derivative.

25 35. A method for enhancing the biological persistence of a neurotoxin of claim 14, wherein a structural modification is fused or added to said neurotoxin.

30 36. The modified neurotoxin of claim 35 wherein said structural modification comprises a leucine-based motif.

37. The modified neurotoxin of claim 35 wherein said structural modification comprises a tyrosine-based motif.

5 38. The modified neurotoxin of claim 35 wherein said structural modification comprises an amino acid derivative.

39. A modified neurotoxin comprising:

10 a botulinum type A neurotoxin including a structural modification, wherein said structural modification is effective to alter a biological persistence of said modified neurotoxin relative to an identical neurotoxin without said structural modification, wherein said
15 structural modification comprises a deletion of amino acids 1 to 8 and 416 to 437 from a light chain of said neurotoxin.

40. A modified neurotoxin comprising:

20 a botulinum type A neurotoxin including a structural modification, wherein said structural modification is effective to alter a biological persistence of said modified neurotoxin relative to an identical neurotoxin without said structural modification, wherein said
25 structural modification comprises substitution of leucine at position 427 for an alanine and leucine at position 428 for an alanine in a light chain of said neurotoxin.

30 41. A method for reducing the biological persistence of a neurotoxin comprising the step of mutating an amino acid of the neurotoxin.

00010346-0004

42. The method of claim 41 which comprises the step of deleting or substituting said amino acid of a leucine-based motif within the neurotoxin.

5

43. The method of claim 41 which comprises the step of deleting or substituting said amino acid from a tyrosine-based motif within the neurotoxin.

10 44. The method of claim 41 which comprises the step of deleting or substituting an amino acid derivative within the neurotoxin.

15 45. A method of treating a condition, comprising a step of administering an effective dose of a modified neurotoxin to a mammal to treat a condition, wherein said modified neurotoxin comprises a neurotoxin including a structural modification, and wherein said structural modification is effective to alter a
20 biological persistence of said neurotoxin.

46. The method of treating a condition of claim 45, wherein said neurotoxin does not comprise a leucine-based motif.

25

47. The method of treating said condition of claim 46, wherein said structural modification includes a biological persistence enhancing component.

30 48. The method of claim 47 wherein said biological persistence enhancing component comprises a leucine-based motif.

49. The method of claim 47 wherein said biological persistence enhancing component comprises a tyrosine-based motif.

5

50. The method of claim 47 wherein said biological persistence enhancing component comprises an amino acid derivative.

10 51. The method of treating said condition of claim 45, wherein said condition comprises a condition selected from the group consisting of a neuromuscular disorder, an autonomic disorder and pain.

15 52. The method of treating said condition of claim 51, wherein treatment of said neuromuscular disorder comprises a step of locally administering an effective amount of said modified neurotoxin to a muscle or group of muscles.

20

53. The method of treating said condition of claim 51, wherein treatment of said autonomic disorder comprises a step of locally administering an effective amount of said modified neurotoxin to a gland.

25

54. The method of treating said condition claim 51, wherein treatment of pain comprises a step of administering an effective amount of said modified neurotoxin to a site of pain.

30

55. The method of treating said condition of claim 51, wherein treatment of pain comprises a step of

administering an effective amount of said modified neurotoxin to a spinal cord.

56. The method of treating said condition of claim 45,
5 wherein said condition is selected from the group
consisting of spasmodic dysphonia, laryngeal dystonia,
oromandibular dysphonia, lingual dystonia, cervical
dystonia, focal hand dystonia, blepharospasm,
strabismus, hemifacial spasm, eyelid disorder, cerebral
10 palsy, focal spasticity, spasmodic colitis, neurogenic
bladder, anismus, limb spasticity, tics, tremors,
bruxism, anal fissure, achalasia, dysphagia,
lacrimation, hyperhydrosis, excessive salivation,
excessive gastrointestinal secretions, pain from muscle
15 spasms, headache pain, brow furrows and skin wrinkles.

57. A modified neurotoxin comprising:

a neurotoxin including a structural modification,
wherein said structural modification is effective to
20 alter a biological activity of said modified neurotoxin
relative to an identical neurotoxin without said
structural modification, and wherein said modified
neurotoxin is structurally different from a naturally
existing neurotoxin.

25

58. The modified neurotoxin of claim 57, wherein said
structural modification is effective to reduce an
exocytosis from a target cell by more than the amount of
the exocytosis reduced from the target cell by an
30 identical neurotoxin without said structural
modification.

59. The modified neurotoxin of claim 57, wherein said structural modification is effective to reduce an exocytosis from a target cell by less than the amount of the exocytosis reduced from the cell by an identical
5 neurotoxin without said structural modification.

60. The modified neurotoxin of claim 58 or claim 59 wherein the exocytosis is exocytosis of a neurotransmitter.

10

61. The modified neurotoxin of claim 57, where the modified neurotoxin exhibits an altered biological activity without exhibiting an altered biological persistence.

15

62. The modified neurotoxin of claim 57, where the modified neurotoxin exhibits an altered biological activity and an altered biological persistence.

20

63. The modified neurotoxin of claim 57, where the modified neurotoxin exhibits an increased biological activity and an increased biological persistence.

25

64. The modified neurotoxin of claim 57, where the modified neurotoxin exhibits an increased biological activity and a reduced biological persistence.

65. The modified neurotoxin of claim 57, where the modified neurotoxin exhibits a decreased biological activity and a decreased biological persistence.

5 66. The modified neurotoxin of claim 57, where the modified neurotoxin exhibits an decreased biological activity and an increased biological persistence.

67. The modified neurotoxin of claim 57 wherein said
10 structural modification comprises a leucine-based motif.

68. The modified neurotoxin of claim 57, wherein a unit
amount of the modified neurotoxin is more efficient to
reduce an exocytosis from a cell than is a unit amount
15 of the naturally existing neurotoxin.